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# Occurrence of Elevated Protoporphyrin Levels in Relation to Lead Burden in Infants

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Simultaneous blood lead (PbB), erythrocyte protoporphyrin (EP), and hematocrit measurements were made semiannually in 232 normal infants from 6 to 24 months of age. The PbB averaged 7 (SD = 5) and ranged from 0 to 64 μg/dl. The incidence of elevated EP, a marker for deranged heme synthesis, was unrelated to PbB at levels below 15 μg/dl but was fourfold greater among the infants with PbB above 15 μg/dl. This relationship persisted even after eliminating the 31 (4%) anemic (hematocrit <33%) samples. The confounding effects of iron deficiency are discussed. 

## INTRODUCTION

Exposure to excessive quantities of lead impairs heme synthesis. Although this was initially recognized in occupational settings among adults (Van den Bergh and Grotepass, 1933), children have been shown to be especially sensitive to this effect (Piomelli *et al.*, 1973). This biochemical alteration occurs in the mitochondria where heme is assembled. When lead poisons this process, the substrate accumulates in peripheral blood as erythrocyte protoporphyrin (EP). Current environmental regulations utilize this marker of biochemical damage as a low-level adverse response of humans to excessive lead exposure (Environmental Protection Agency, 1977).

Recently, Piomelli and his colleagues reported a threshold of lead's effect on EP (1982). In their survey of 2004 children aged 2 to 12 years with a mean blood lead (PbB) of 17 μg/dl, the incidence of elevated EP was nearly constant when PbB was below 15–18 μg/dl, but rose progressively at higher PbB levels. The present study was designed to assess in younger infants with generally lower PbB the relationship between current levels of lead exposure and EP.

## MATERIALS AND METHODS

From a survey of umbilical cord PbB from 11,837 consecutive births at the Boston Lying-In Hospital between September 1979 and April 1981, 249 children were enrolled for a study of lead and infant development. The subjects were drawn equally from the highest, lowest, and centermost deciles of PbB. To be eligible for enrollment the child must also have been expected to live within the greater Boston area (inside Route 128) for the next 2 years in an English-speaking household, and to be free of serious medical conditions. Informed consent was



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obtained from the parents in every case. After 2 years 201 infants remained in this study. In general, the families were white (87%), well educated (mean maternal schooling, 14.5 years), living in intact families (88% with both parents), and mature (mean maternal age, 30 years).

After scrupulously cleaning the hand and finger, capillary samples of blood were collected at 6, 12, 18, and 24 months of age. PbB was measured in duplicate by anodic stripping voltammetry using an exchange medium (ESA Model 3010, Bedford, Mass.). Each batch of samples was accompanied by aliquots of pooled standardized blood samples, and all values were calculated by comparisons to amperage peaks from a gravimetrically prepared lead solution. This laboratory participated in a series of blind comparisons coordinated by the West Allis Memorial Hospital. Using this assay, the mean absolute difference between our reported values and the reference method for 25 samples was 2.9 (SD = 2.8) μg/dl. For the nine samples with PbB less than 50 μg/dl the mean absolute difference was 2.0 (SD = 1.2). The mean difference between duplicate samples was 1 μg/dl.

EP was measured with an ESA Model 4000 hematofluorometer as the zinc species. In monthly interlaboratory blind trials with 111 samples sponsored by the Center for Disease Control, our method produced values with an average absolute difference from the reference values of 8 (SE = 1) μg/dl, a difference of 6 for the 53 samples with EP less than 100, and a difference of 3 for the 8 samples with EP under 50, the range encountered in our sample. Hematocrit (Hct) was measured by the centrifugation of peripheral blood in a capillary tube with a Clay–Adams Microcrit.

For 21 of the 860 collections (2.4%) either PbB or EP was not measured because of limited sample volume. Table 1 shows the number of determinations of EP and PbB from these infants at different ages.

## RESULTS

The mean PbB was 7 (SD = 5) μg/dl with a range of 0 to 64. Only five samples (0.6%) had values exceeding 30. EP averaged 17 (SD = 17) μg/dl and ranged from 0 to 253, a highly skewed distribution (kertosis = 72). Only 10% had EP of 30 or more. Table 1 shows the mean values and ranges of PbB and EP of the children at the four different ages. A $\chi^2$ test revealed no significant differences in the incidence of elevated EP among the four age categories ($\chi^2(1) = 5.7$, $P > 0.1$). The pair-wise values of EP and PbB correlated very well (Spearman, nonparametric $r = 0.15$, $N = 839$, $P < 0.005$).

The incidence of elevated EP was examined by considering two upper limits of normal. A total of 6.2% of the samples exceeded the mean + 1 SD (35 μg/dl), and 1.8% exceeded the mean + 2 SD (52 μg/dl). Among children with PbB below 15 μg/dl, the incidence of elevated EP is at a constant or "natural" frequency (Table 2). However, with higher PbBs, a dose (PbB)–response (elevated EP) relationship is evident. If the cases are divided by PbB above and below 15 μg/dl, based on the previously reported threshold for PbB (Piomelli *et al.*, 1982), the incidence of EP > 34 is significantly elevated in children with higher (>15) PbB levels ($\chi^2(1) = 5.9$, $P < 0.02$).

The confounding effect of iron deficiency anemia was considered by excluding

TABLE 1
BLOOD LEAD (PbB) AND PROTOPORPHYRIN (EP) LEVELS IN INFANTS AT DIFFERENT AGES

| | | Age (months) | | | |
|---|---|---|---|---|---|
| | | 6 | 12 | 18 | 24 |
| PbB | Number | 221 | 204 | 213 | 202 |
| | Mean | 6.2 | 7.8 | 6.8 | 7.0 |
| | Range | 0–49 | 0–31 | 0–27 | 0–64 |
| EP | Number | 230 | 214 | 210 | 202 |
| | Mean | 21 | 20 | 16 | 12 |
| | Range | 0–128 | 0–253 | 0–227 | 0–152 |
| | Number > 34 μg/dl | 15 (7)[a] | 16 (8) | 14 (7) | 7 (3) |
| | Number > 52 μg/dl | 5 (2) | 5 (2) | 2 (1) | 3 (1) |

[a] Values in parentheses are percentages.

the few (3.7%) samples with Hct < 33%. This restriction did not alter the pattern between PbB and elevated EP. Anemic children did tend to have elevated EP; 74% had EP above 30, compared with 10% among nonanemic ones. Mean PbB in the anemic subgroup, 6.95 μg/dl, was nearly the same as in the other children, 7.03 μg/dl. Excluding anemic infants did eliminate only 3 of the 15 cases of EP < 52, none having PbB > 10.

## DISCUSSION

In this study of children during their first 2 years of life, PbB greater than about 15 μg/dl was associated with a greater incidence of elevated EP. This finding supports the observations of Piomelli *et al.* (1982) whose larger survey of older children demonstrated a threshold of PbB's effect on EP near 16.5 μg/dl.

TABLE 2
THE OCCURRENCE OF "ELEVATED" EP AMONG CHILDREN CLASSIFIED BY PbB CATEGORY[a]

| | Number of samples[b] | | | | | |
|---|---|---|---|---|---|---|
| | Among all subjects | | | Excluding anemia | | |
| PbB category | Total | EP > 34 | EP > 52 | Total | EP > 34 | EP > 52 |
| 0 to 1.9 | 165 | 10 (5) | 2 (1) | 155 | 9 (6) | 2 (1) |
| 2.0 to 4.9 | 210 | 6 (3) | 3 (1) | 207 | 5 (1) | 3 (1) |
| 5.0 to 9.9 | 263 | 21 (8) | 3 (1) | 254 | 20 (8) | 3 (1) |
| 10 to 14.9 | 102 | 3 (3) | 2 (2) | 96 | 1 (1) | 0 (0) |
| 15 to 19.9 | 63 | 7 (11) | 3 (5) | 60 | 6 (10) | 2 (3) |
| 20 + | 36 | 5 (14) | 2 (6) | 36 | 5 (14) | 2 (6) |
| Total | 839 | 52 (6) | 15 (2) | 808 | 46 (6) | 12 (1) |

[a] All EP and PbB units are expressed as micrograms per deciliter.
[b] Values in parentheses are percentages.

The observed threshold value of PbB above which EP becomes more frequently "elevated" is dependent on the definition of "elevated" in this study population. Using the higher EP cutoff of 52 (mean + 2 SD) results in a threshold in the 15–20 range of PbB, while a lower cutoff of 34 (mean + 1 SD) yields a threshold in the 10–15 range. This apparent dependence of the PbB threshold upon the choice of EP cutoff was not evident in the older, larger, and more lead-burdened population studied by Piomelli *et al.* (1982). They found the PbB threshold to be 15–18, whether they used 33 or 53 as their defined cutoff for "elevated" EP.

Piomelli and his colleagues (1982) chose to exclude infants less than 2 years of age from their report because iron deficiency, common at that age, might have confounded any lead effect on EP. We attempted to remove any confounding by eliminating cases with Hct < 33%, those with iron deficiency severe enough to cause anemia. Removing anemic subjects did not alter the relationship between EP and PbB (Table 2). However, since we did not measure iron stores directly, we do not know if milder iron deficiency without anemia would alter the relationship between lead and elevated EP. Such a deficiency would have to be more prevalent in the higher lead categories, but severe iron deficiency is equally prevalent regardless of lead burden. Hemolytic anemia, in addition to excessive lead and iron deficiency, can also elevate protoporphyrin levels. None of our subjects had sickle cell disease or thalassemia, and none was feverish at the time of collections.

Current environmental standards for lead exposure are designed to protect the population, especially the young, from toxic manifestations. Our findings support PbB levels near 15 μg/dl as the highest no-effect level for infants.

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*Addendum.* A further review of the data reveals patterns of increasing intrapersonal stability of EP with age, as shown in the table below. The subjects of this study were sampled repeatedly and Spearman rank correlations of individuals' EP and PbB values at 6-month intervals were calculated. An $r$ of 1.0 would be a perfectly preserved ranking of the children over the time span. At each epoch EP levels are more persistent than PbB. Both EP and PbB rankings become more stable with maturity.

INTRAPERSONAL STABILITY OF BLOOD LEAD AND PROTOPORPHYRIN

| | Spearman correlation coefficients ($r$) between ages (months) | | | |
|---|---|---|---|---|
| Substance in blood | Birth–6 | 6–12 | 12–18 | 18–24 |
| PbB lead | 0.10 | 0.19* | 0.41** | 0.61** |
| EP protoporphyrin | | 0.37** | 0.58** | 0.66** |

*Note.* 197 to 212 matched pairs from the same child at each span.
* $P < 0.01$.
** $P < 0.001$.

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